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14. ABSTRACT

The purpose of this research is to reveal the relationship between assembly of the primary cilium and prostate cancer by investigating two centrosomal proteins, pericentriolar material 1 (PCM1) and Mind bomb 1 (Mib1), and to test the possibility that these proteins can serve as robust **prostate cancer biomarkers** and, potentially, **targets for drug discovery**. The primary cilium serves as a cellular antenna, and this organelle inhibits cell proliferation. The loss of this key signaling organelle was reported in various cancers, including prostate cancer. Therefore, we hypothesize that the absence of a primary cilium can potentially trigger cell proliferation and prostate cancer development. Pericentiolar material 1 is essential for ciliogenesis, and the *PCM1* gene is deleted in ~15% of prostate cancers. Our results indicate that ablation of pericentriolar material 1 leads to aberrant expression of its interacting partner, Mind bomb 1, an enzyme that is a negative regulator of ciliogenesis. Based on these data, we hypothesize that elevated levels of Mind bomb1 provoked by pericentriolar material 1 depletion in prostate cancer promote abnormal cell growth and malignancy by preventing the assembly of cilia. To test this hypothesis, we will investigate the impact of pericentriolar material 1 deletions in prostate cancer and determine whether there is a correlation between increased Mind bomb 1, the loss of cilia, and the stage of prostate tumor progression. Next, we will test whether pericentriolar material 1 depletion and aberrant levels of Mind bomb 1 promote prostate cancer development. Finally, we will investigate whether Mind bomb 1 removal induces ciliogenesis and inhibits growth of prostate cancer to determine the suitability of Mind bomb 1 as a target of anti-cancer drugs.

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1. INTRODUCTION:

The purpose of this research is to reveal the relationship between assembly of the primary cilium and prostate cancer by investigating two centrosomal proteins, pericentriolar material 1 (PCM1) and Mind bomb 1 (Mib1), and to test the possibility that these proteins can serve as robust **prostate cancer biomarkers** and, potentially, **targets for drug discovery**. The primary cilium serves as a cellular antenna, and this organelle inhibits cell proliferation. The loss of this key signaling organelle was reported in various cancers, including prostate cancer. Therefore, we hypothesize that the absence of a primary cilium can potentially trigger cell proliferation and prostate cancer development. PCM1 is essential for ciliogenesis, and the *PCM1* gene is deleted in ~15% of prostate cancers. Our results indicate that ablation of PCM1 leads to aberrant expression of its interacting partner, Mib1, an enzyme that is a negative regulator of ciliogenesis. Based on these data, we hypothesize that elevated levels of Mib1 provoked by PCM1 depletion in prostate cancer promote abnormal cell growth and malignancy by preventing the assembly of cilia. To test this hypothesis, we will investigate the impact of PCM1 deletions in prostate cancer and determine whether there is a correlation between increased Mib1, the loss of cilia, and the stage of prostate tumor progression. Next, we will test whether PCM1 depletion and aberrant levels of Mib1 promote prostate cancer development. Finally, we will investigate whether Mib1 removal induces ciliogenesis and inhibits growth of prostate cancer to determine the suitability of Mib1 as a target of anti-cancer drugs. Collectively through our research proposal, we will be able to understand a novel regulatory pathway in the formation of primary cilia and its implications in prostate cancer development. These efforts will ultimately allow us to devise innovative and effective therapeutic approaches against malignant prostate cancer. First, our investigation may allow us to find **new bio-markers** to detect cancer. In addition, since we are examining a "druggable" protein with enzymatic activity, our results could ultimately allow discovery of **potential anti-cancer drugs** for effective therapeutic approaches against malignant prostate cancer, which is a leading, fatal disease threatening the male population worldwide.

2. KEYWORDS:

Primary cilium, prostate cancer, PCM1, Mib1

3. ACCOMPLISHMENTS:

What were the major goals of the project? What was accomplished under these goals?

Training-Specific Tasks:
Subtask 2: Present research at the monthly department group meetings
Completed
Subtask 3: Attend a national scientific meeting in relevant scientific field
I attended the Cold Spring Harbor Asia conference on Cilia & Centrosomes and presented my work as poster "Tethering of an E3 ligase by PCM1 regulates the abundance of centrosomal KIAA0586/Talpid3 and promotes ciliogenesis".
Milestone Achieved: Presentation of project data at a national meeting

Specific Aim 1 Major Task 1: To investigate whether PCM1 deletion in prostate cancer is correlated with an increase in Mib1 and the loss of cilia
Subtask 1: Measurement of PCM1 and Mib1 expression in normal prostate and prostate

cancer tissue arrays.

Results: We studied the expression of PCM1 and Mib1 proteins in human prostate cancer (PCa) tissue samples by immunohistochemical analysis of a human PCa TMA derived from a cohort of PCa patients ($n = 131$) in various clinicopathological groups. TMAs were obtained from the DOD-sponsored PCBN repository at Johns Hopkins University. In collaboration with prostate cancer pathologists and a biostatistician, we first examined the expression of PCM1 and Mib1 in normal and malignant prostatic epithelial cells in prostate tissue. The mean protein intensity of cytoplasmic PCM1 and Mib1 were significantly increased in PCa compared to the adjacent normal tissues ($p < 0.001$) (Figure 1). We did not observe a significant association between the change in Mib1 and PCM1 expression level with Gleason primary score (Figure 2), Gleason sum score (Figure 3) or prostate cancer stage (Figure 4). Then we compared expression of PCM1 and Mib1 proteins in non-hormone resistant tumor groups to hormone-resistant tumor group and found no significant difference (Figure 6 and Table 1). Finally, we examined the expression of PCM1 and Mib1 in normal and high grade PIN epithelial cells. **The mean protein intensity of cytoplasmic PCM1 and Mib1 were significantly increased in high-grade prostate intraepithelial neoplasia (HGPIN) compared to the adjacent normal tissues** ($p < 0.001$) (Figure 5).

Figure 1. Protein intensity of PCM1 and Mib1 in normal and PCa tissues. Paired Wilcox rank test. * P<0.05.
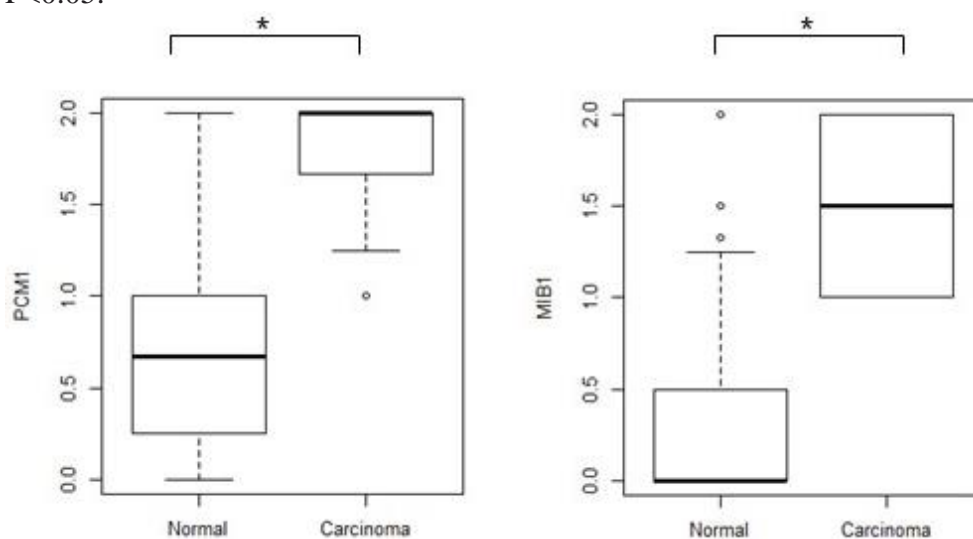
Figure 2. Simple linear regression model was used to study association of protein expression changes (tumor - normal) and Gleason-Primary score.
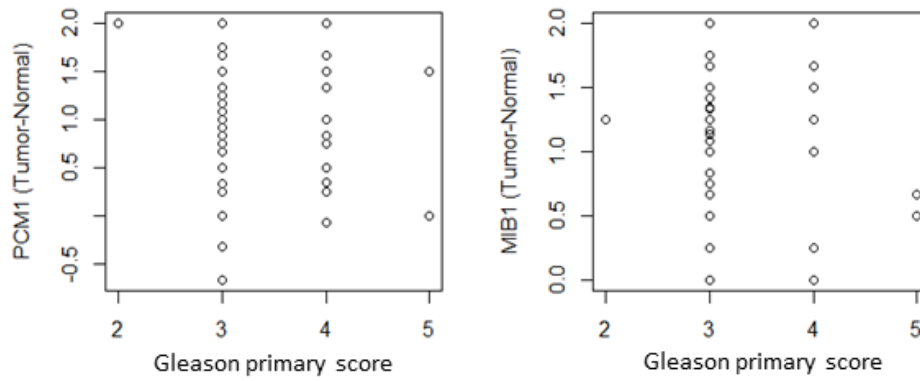

Figure 3. Simple linear regression model was used to study association of protein expression changes (tumor - normal) and Gleason-Sum score.
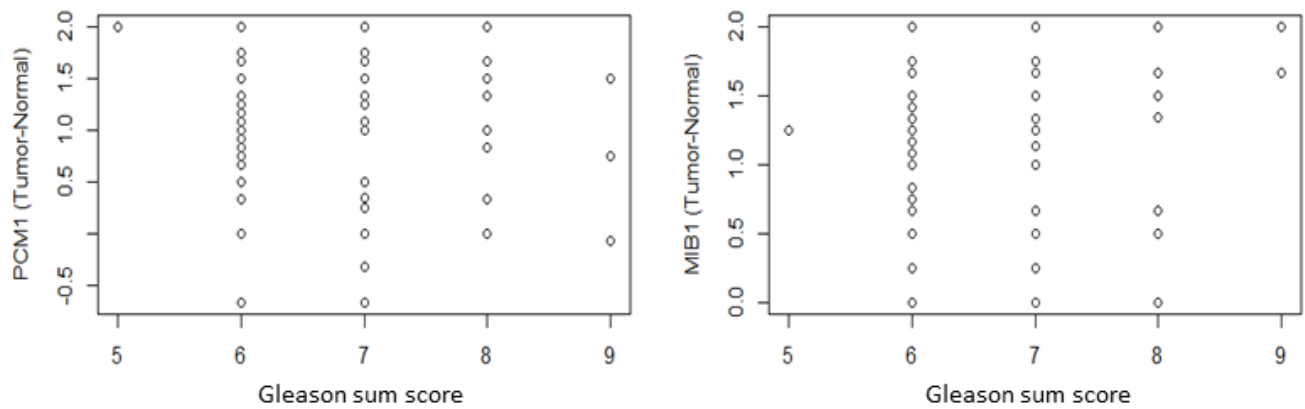
Figure 4. Analysis of variance (ANOVA) was used to test the association between protein expression changes and TNM stage.
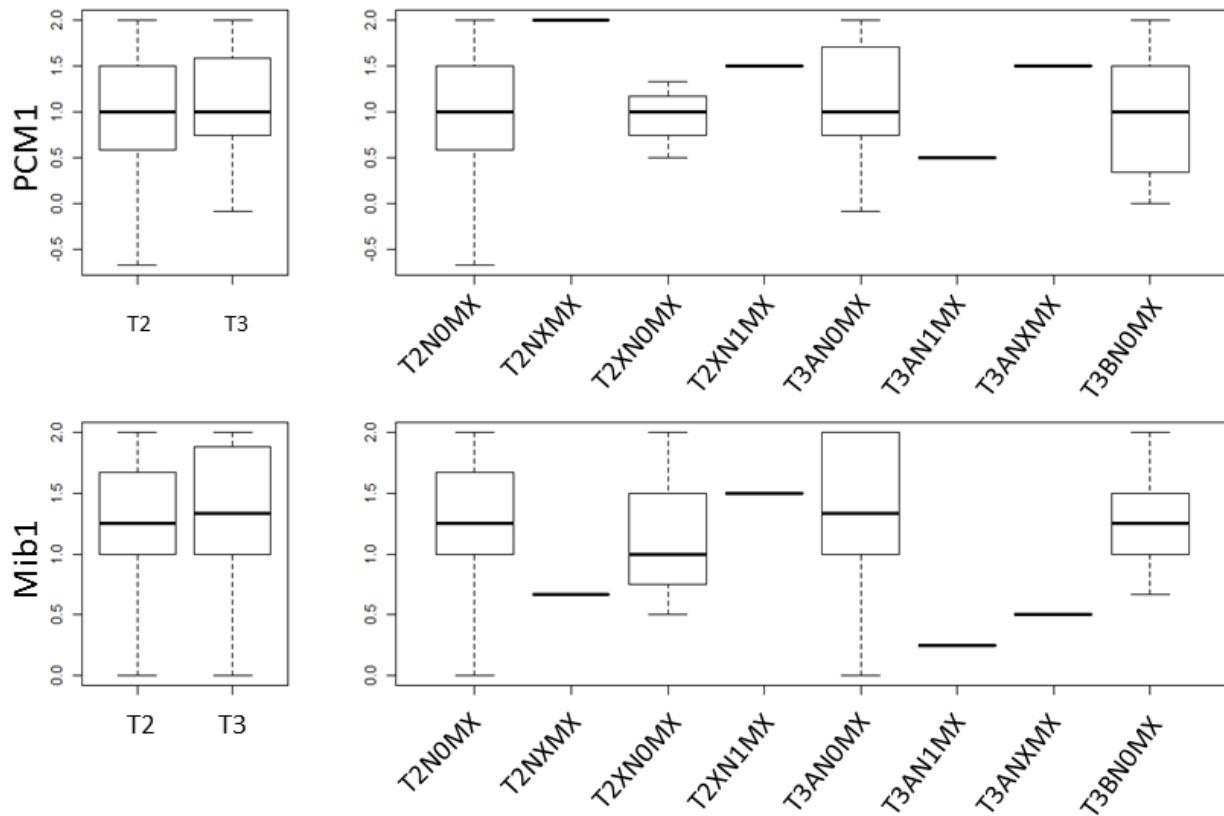

Figure 5. Protein intensity of PCM1 and Mib1 in normal and HGPIN tissues. Paired Wilcox rank test. * P<0.05.
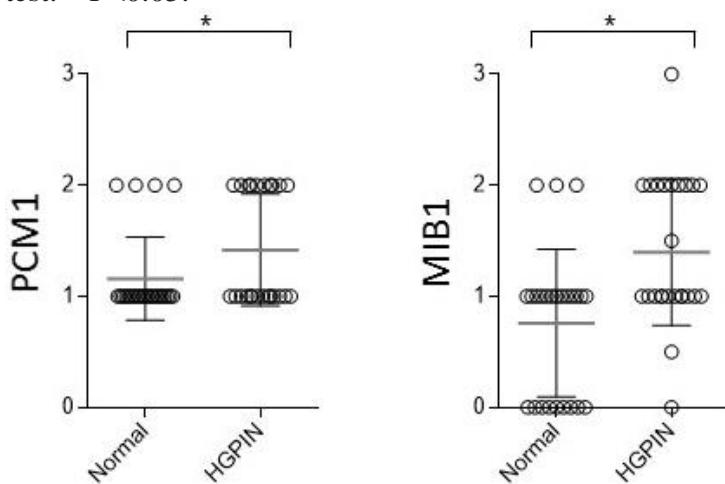
Figure 6. Comparison of non-resistant tumor groups and resistant tumor group (i.e., group I, III, IV vs group II).
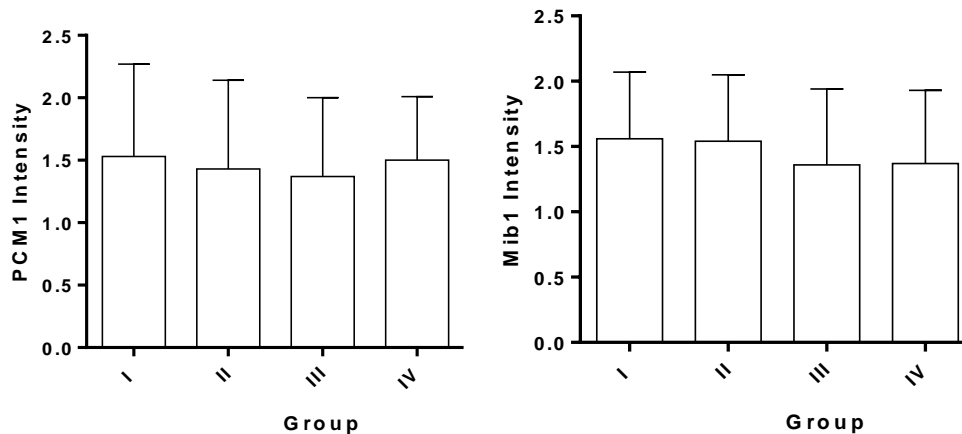

Table 1. Sample size summary.

Group	Type	Sample size
I	Tumor from Hormone Naïve, TURP	15
II	Tumor from Hormone Refractory, TURP	40
III	Tumor from Neo Adjuvant, Radicals	27
IV	Tumor from No therapy, Radicals	28

Subtask 2: Measurement of ciliation and proliferation in PCM1 positive and negative prostate cancer tissue and cell lines

Results: We hypothesize that epithelial cells in tumors will have lost their primary cilia. Therefore, we will examine the correlation between PCM1, Mib1 expression levels and ciliation by co-staining CK5, acetylated tubulin (Ace-Tubulin, a marker for cilia), and γ-Tubulin (a marker for centrosomes) in TMAs. We developed and optimized a triple-staining protocol of CK5, Ace-Tubulin, and γ-Tubulin by staining pilot prostate tissue TMAs (Figure 7). Our application for TMAs has been approved by PCBN and we will start the staining as soon as we get the TMAs. We also measured the expression level and ciliation potential of PCM1 and Mib1 in normal, benign prostate epithelia cell lines and PCa cell lines. Consistent with our findings in TMAs, PCM1 was found to be over-expressed in certain PCa and benign cell lines (NEI-8, RC165 and BPH-1) compared to a normal prostate cell line (RWPE-1) and stem cell line, WPE. Mib1 was also over-expressed in certain PCa and benign cell lines (LnCaP, RC165 and BPH-1) compared to normal prostate cell lines (RWPE-1 and WPE). Moreover, Pca and benign cell lines (LnCaP, LnCaP-AI, NEI-8, RC165 and BPH-1) have lower ciliation rates compared to normal prostate cell lines (RWPE-1 and WPE, Figure 8).

Milestone(s) Achieved: Correlate over-expression of Mib1 and PCM1 with stage of tumor

6

Figure 7. Triple-staining of prostate tissue for CK5, Ace-tubulin, and γ-tubulin. Staining of pilot TMA is shown, and each marker is indicated at bottom right of each panel.
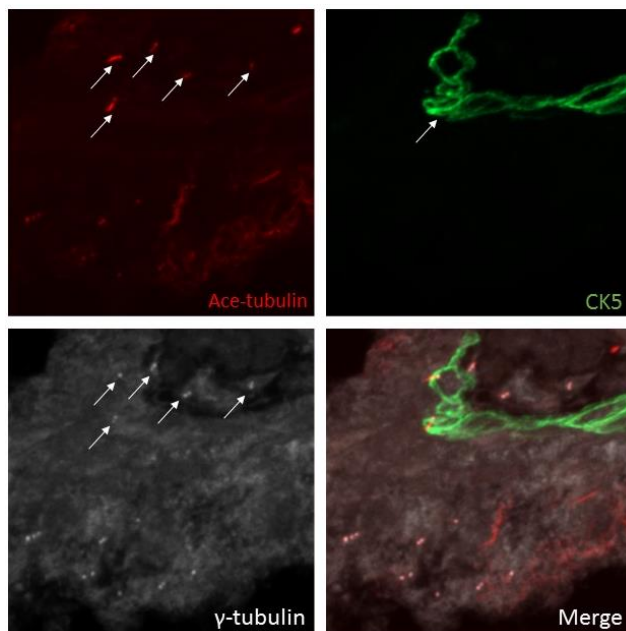

Figure 8. Measurement of ciliation and protein level of PCM1 and Mib1 in prostate cell lines. "++", "+" and "-" indicate ~20%, ~10% and ~0% of cells were ciliated respectively.
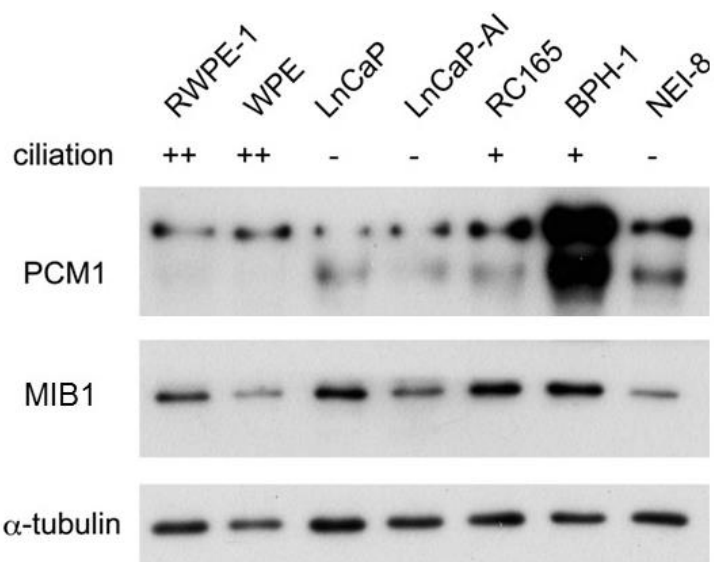
Specific Aim 2: To investigate the impact of PCM1 and Mib1 loss and Mib1 over-expression in prostate cancer development

Major Task 2: The impact of deleting *PCM1* or Mib1 in prostate cell lines and zebrafish xenograft model.

Subtask 1: Establishment of prostate normal and cancer cell lines depleted for PCM1 or Mib1

Subtask 2: Investigation of ciliation, proliferation and malignancy of the cell lines and tumor

formation.

Since our TMA and cell lines data show that Mib1 protein is over-expressed in prostate tumors, our work will have considerable impact if we are able to demonstrate that deleting of Mib1 in Pca or benign cell lines rescues ciliogenesis and inhibits cell growth. We chose LNCap and RC165, which have higher Mib1 expression level compared to normal prostate cell lines (Figure 8), to test our hypothesis. The establishment of Mib1 knockout LNCap and RC165 cell lines is in progress. We will test ciliogenesis, cell growth, and tumor formation using these cell lines.

Major Task 3: The impact of stably over-expressing Mib1 in prostate cell lines and zebrafish xenograft model.

Since our TMA and cell lines data show that Mib1 protein is over-expressed in prostate tumors, our work will have considerable impact if we are able to demonstrate that overexpression of Mib1 inhibits ciliogenesis and promotes cell growth. To further test this possibility, we ectopically expressed Mib1 using a doxycycline (Dox)-inducible system in normal prostate cell lines, RWPE1 and WPE, and selected stable cell lines (Figure 9). We found that over-expression of Mib1 could indeed inhibit the ciliation rates of normal prostate cell lines (Figure 10).

Figure 9. Establishment of normal prostate cell lines stably over-expressing Mib1.
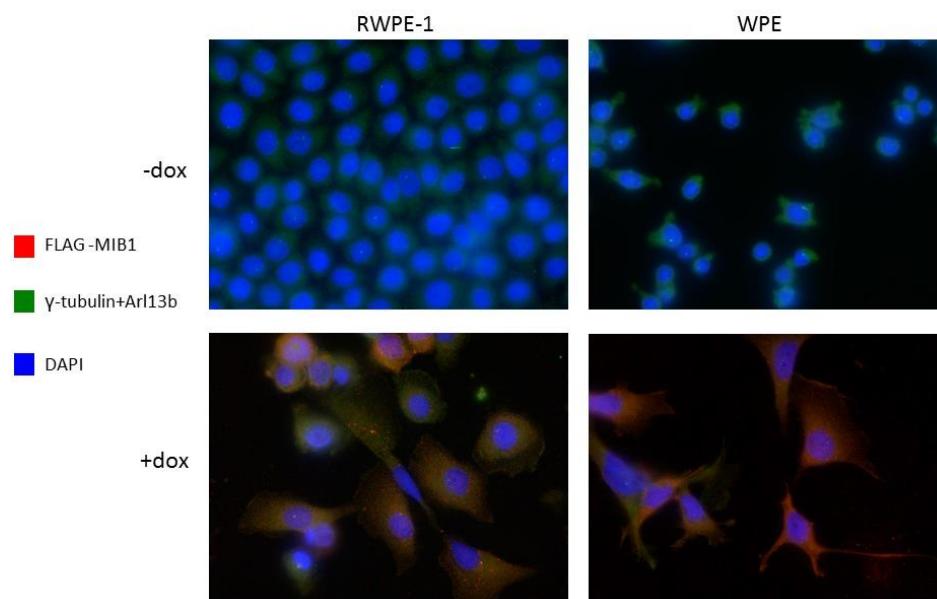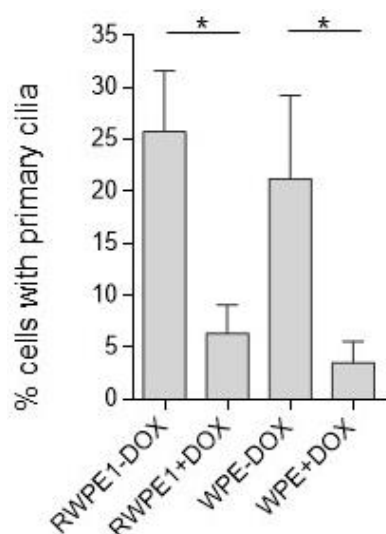
Figure 10. Over-expression of Mib1 inhibits ciliogenesis in normal prostate epithelial cell lines.

Mentoring-Specific Tasks: Major Task 1: Mentoring and discussion about progression in prostate cancer research.

Subtask 1: Weakly meeting with mentor, Dr. Dynlacht

Completed

Subtask 2: Monthly meeting with mentor, Dr. Dynlacht and co- mentor, Dr. Lee

Completed

What opportunities for training and professional development has the project provided?

I am working at the NYU School of Medicine (NYUSoM) Cancer Institute as a postdoctoral fellow under the supervision of Dr. Dynlacht and my co-mentor, Dr. Peng Lee, an expert in prostate pathology and cancer. My work seeks to understand the relationship between primary cilia dysfunction and prostate cancer. This fellowship allowed me to acquire basic knowledge and technical expertise related to the biology of the prostate and prostate cancer biology, including approaches to study proliferation, invasion, migration, anchorage-independent growth, and apoptosis in Dr. Peng Lee's laboratory. As time permits, I will extend the proposed research by introducing genetically engineered mouse models of prostate cancer, through which I will acquire essential expertise with mouse models to study prostate cancer. Dr. Cory Abate-Shen, our collaborator, will provide additional training opportunities and instruction. In addition, I learned biochemical and cell biological approaches in the Dynlacht laboratory, and Dr. Dynlacht provided mentoring and advice needed to proceed to the next, independent stage of my career. Most importantly, I will learn from both mentors critical thinking, experimental design, and grant writing to prepare for my independent research. In summary, this program would provide me with an outstanding opportunity to advance toward my career goals.

How were the results disseminated to communities of interest?

Manuscript in progress.

What do you plan to do during the next reporting period to accomplish the goals?

We will finish the experiments we proposed for the second year. Specifically, we will engineer normal and cancerous prostate cell lines overexpressing or depleted for PCM1 or Mib1 and investigate ciliation, proliferation, and malignancy of these cell lines and tumor formation in mice.

These works will be presented at domestic meetings.

4. IMPACT
What was the impact on the development of the principal discipline(s) of the project?
Primary cilia play a repressive role in regulating cell proliferation and are frequently absent in numerous types of cancer, including prostate cancer. The purpose of this research is to reveal the relationship between assembly of the primary cilium and prostate cancer by investigating two centrosomal proteins, pericentriolar material 1 (PCM1) and Mind bomb 1 (Mib1). Further, defining the role and function of cilia during the course of prostate cancer malignancy will instigate novel insights into therapeutic approaches against prostate cancer. These insights will enable us to understand novel regulatory pathways in the formation of primary cilia and their implications for prostate cancer development. Our work may have major impacts on two fronts. First, our investigation may allow us to find new bio-markers (PCM1 and Mib1) to detect cancer. In addition, since we are examining a "druggable" protein with enzymatic activity, our results could ultimately allow discovery of potential anti-cancer drugs (Mib1) for effective therapeutic approaches against malignant prostate cancer, which is a leading, fatal disease threatening the male population worldwide.

What was the impact on other disciplines?
N/A

What was the impact on technology transfer?

If our research is successful, it could define PCM1 and Mib1 as important biomarkers and, potentially, targets of anti-cancer drugs for prostate cancer, thus relating to the Overarching Challenge of Diagnosis and Therapy for prostate cancer. Importantly, since Mib1 is an enzyme, it is a possible actionable target. If successful, in the future, we will work with our Office of Technology licensing to commence collaborations with potential pharmaceutical partners.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:
Changes in approach and reasons for change

No changes in approach/nothing to report.
Actual or anticipated problems or delays and actions or plans to resolve them

None anticipated/nothing to report.
Changes that had a significant impact on expenditures

None.
Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None.

6. PRODUCTS:
Publications, conference papers, and presentations

I attended the Cold Spring Harbor Asia conference on Cilia & Centrosomes and presented my work as poster "Tethering of an E3 ligase by PCM1 regulates the abundance of centrosomal KIAA0586/Talpid3 and promotes ciliogenesis".

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

N/A

Inventions, patent applications, and/or licenses
N/A
Other Products
N/A

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

1. Name: Lei Wang
Role: PI
Nearest person month worked: 12 months
Contributions to Project: Dr. Wang performed all the experiments.

2. Name: Brian Dynlacht
Role: Mentor
Nearest person month worked: 1
Contribution to Project: Dr. Dynlacht provided mentoring and advice on cell biology and biochemistry.
3. Name: Peng Lee
Role: Co-mentor
Nearest person month worked: 1

Contribution to project: Dr. Lee provided mentoring and advice on prostate cancer and assisted in acquiring and analyzing patients samples.

8. SPECIAL REPORTING REQUIREMENTS
Nothing to report.

9. APPENDICES:
Nothing to report.